

## **Guidelines on the management of drug-induced immune and secondary autoimmune, haemolytic anaemia**

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### **KEYWORDS**

Autoimmune haemolytic anaemia, drug induced immune haemolytic anaemia, Evans syndrome, cancer, infection, systemic lupus erythematosus, common variable immunodeficiency, ulcerative colitis, transplantation.

## **SCOPE**

The objective of this guideline is to provide healthcare professionals with guidance on the management of patients with secondary autoimmune haemolytic anaemia (AIHA). The guidance may not be appropriate to every patient and in all cases individual patient circumstances may dictate an alternative approach.

## **Methodology**

### ***Literature review details***

Recommendations are based on the systematic review of published English language literature from January 1960 to October 2015 (see [supplementary appendix 1](#) for further details).

The Grading of Recommendations Assessment, Development and Evaluation (GRADE) nomenclature was used to evaluate levels of evidence and to assess the strength of recommendations. The GRADE criteria are specified in the British Committee for Standards in Haematology (BCSH) guidance pack ([http://www.bcshguidances.com/BCSH\\_PROCESS/42\\_EVIDENCE\\_LEVELS\\_AND\\_GRADES\\_OF\\_RECOMMENDATION.html](http://www.bcshguidances.com/BCSH_PROCESS/42_EVIDENCE_LEVELS_AND_GRADES_OF_RECOMMENDATION.html)) and the GRADE working group website <http://www.gradeworkinggroup.org>

### ***Working group membership***

The guideline group was selected to be representative of UK-based experts in the diagnosis and management of AIHA.

### ***Review***

Review of the manuscript was performed by the BCSH General Haematology Task Force, BCSH Executive Committee and then a sounding board of the

British Society for Haematology (BSH). This comprises 50 or more members of the BSH who have reviewed this Guidance and commented on its content and applicability in the UK setting.

## **BACKGROUND**

AIHA is a decompensated acquired haemolysis caused by the host's immune system acting against its own red cell antigens. The incidence is 1 per 100,000/year and approximately half are secondary to an associated disorder. Serologically, cases are divided into warm, cold (cold haemagglutinin disease and paroxysmal cold haemoglobinuria) or mixed AIHA (Table I). Cases of drug-induced immune haemolytic anaemia (DIIHA) make up about 10% of the total when included in series of patients with AIHA (Liesveld *et al*, 1987; Petz & Garratty, 1980; Sokol *et al*, 1992).

The presenting features, investigations and diagnostic approach to AIHA are covered in recent BCSH guidelines on primary AIHA. AIHA can be diagnosed when there is laboratory evidence of haemolysis, a positive direct antiglobulin test (DAT) and clinical evaluation has excluded an alternative cause (e.g. DIIHA, haemolytic transfusion reaction or post transplantation alloimmune haemolysis).

Some general strategies taken in primary AIHA are also applicable. Hence if DIIHA is suspected, relevant medication should be stopped. Patients should receive folic acid. Haemolysis is a risk factor for venous thrombosis and patients should be risk assessed for thromboprophylaxis. Patients receiving steroids should also be risk assessed for treatment to prevent glucocorticoid-induced osteoporosis and gastrointestinal bleeding. Patients in whom anaemia is life threatening should be transfused with ABO, Rh and Kell matched red cells rather than waiting for full compatibility testing. An underlying disorder or its treatment may however influence other requirements, for example the need for an irradiated product following treatment with fludarabine.

Secondary AIHA broadly encompasses conditions that appear to occur in association with AIHA. The strength and significance of the association varies and conditions may represent two parts of a complex immune mediated disorder (Sokol & Hewitt, 1985). Often, the associated condition should be treated in its own right along current best practice guidelines and successful treatment may (but does not always) improve the AIHA. When the associated condition appears inactive or would not otherwise require treatment, management of AIHA can usually proceed along similar lines to primary AIHA, although cases must be individualised.

The distribution of secondary AIHA varies according to the population studied but approximately half are associated with haematological malignancy, a third with infection and a sixth with collagen vascular disorders (Dacie & Worlledge, 1969; Pirofsky & Bardana, Jr., 1974; Sokol *et al*, 1992; Vaglio *et al*, 2007). Most cases are warm but secondary cold haemagglutinin disease (CHAD) is also reported. Some common or important associations are considered below.

## **NEOPLASMS**

### **Haematological malignancy**

AIHA is reported in patients with a wide range of haematological malignancies, most frequently in CLL and non-Hodgkin lymphoma (NHL). Patients with AIHA are also at an increased risk of subsequently developing NHL, myeloma, CLL or myeloid malignancies. A positive DAT without haemolysis is more frequent than AIHA in CLL (Dearden *et al*, 2008) and myelodysplastic syndromes (MDS) (Sokol *et al*, 1989). Diagnosis of secondary AIHA can be complex because the anaemia may be multifactorial, the LDH affected by disease progression or liver dysfunction and a reticulocyte response prevented by marrow infiltration

(Dearden, 2008). A bone marrow biopsy may therefore be required as part of the assessment. CMV reactivation and parvovirus B19 infection should be excluded.

Of 73 patients receiving intravenous immunoglobulins (IVIg) for warm AIHA, 40% responded and secondary associations (28 had lymphoma or CLL) did not predict outcome (Flores *et al*, 1993). IVIg may therefore be considered as a rescue option in patients with a haematological malignancy and poorly controlled warm AIHA.

### ***Chronic Lymphocytic Leukaemia***

AIHA occurs in 5–10% of patients with CLL (Dearden *et al*, 2008; Mauro *et al*, 2000) and its management is addressed in recent BCSH guidelines (Oscier *et al*, 2012).

### ***Non-Hodgkin Lymphoma***

AIHA may precede NHL but is usually reported at or following diagnosis. Overall, AIHA occurs in 2–3% of NHL patients but rates of 13–19% are reported in angioimmunoblastic T-cell lymphoma (Lachenal *et al*, 2007). In a literature review, complete remission was achieved in 39/56 with anti-lymphoma therapy but only 8/34 with steroids or immunoglobulins (Hauswirth *et al*, 2007). For B-cell NHL, treatment with rituximab as a single agent or combined with chemotherapy was often a successful approach (Hauswirth *et al*, 2007), as was splenectomy for splenic marginal zone lymphoma. Small patient numbers and heterogeneous regimens make treatment recommendations difficult and NHL type and remission status will influence treatment selection. NHL in complete remission and low grade NHL not otherwise requiring treatment favour an initial AIHA rather than lymphoma directed approach.

In recent BCSH guidelines on Waldenström macroglobulinaemia, rituximab-based therapy was recommended for symptomatic secondary CHAD, with the addition of fludarabine to be considered for those with adequate performance status and renal function (Owen *et al*, 2014).

### ***Hodgkin lymphoma***

AIHA occurs in 0.2% of patients with Hodgkin lymphoma (HL) (Varoczy *et al*, 2002;Xiros *et al*, 1988). In a systematic review (Lechner & Chen, 2010) 34 cases were identified; 29 of which were at an advanced stage. Eight presented with AIHA 5 months to 21 years prior to HL, 18 concurrently and 8 at time of relapse. Two IgA AIHA cases presented during CR of HL and were steroid responsive. In the remaining patients, although some responded to steroids or splenectomy, all treated with anti-lymphoma therapy responded, usually completely.

#### **Hodgkin lymphoma: Recommendations**

- **First line therapy for AIHA associated with HL is anti-lymphoma therapy (1C)**
- **Patients presenting with AIHA during remission of HL should be assessed carefully for relapse (1A). If the patient is in confirmed complete remission, treatment should be as for primary AIHA (2C).**

### **Solid organ neoplasia**

#### ***Benign conditions***

The most frequently reported associations are ovarian teratoma and thymoma. Rarely, other non-malignant ovarian tumours and non-ovarian teratomas have also been reported (Buonanno *et al*, 1984;Goyal *et al*, 2010;Payne *et al*, 1981).

*Ovarian teratoma* AIHA is a rare association and was not reported in a series of 517 teratomas (Comerci, Jr. *et al*, 1994). Case reports show that patients respond poorly to steroids or splenectomy but AIHA consistently resolves with resection of the tumour.

#### **Ovarian teratoma: Recommendations**

- **First line therapy is surgical resection (1C).**
- **When resecting the tumour, concomitant splenectomy is not indicated (1C)**

*Thymoma* warm AIHA is a rare association and in the majority, occurs at presentation with, or after diagnosis of, thymoma. Although most cases are steroid responsive, the majority of patients proceed to thymectomy, which usually leads to prompt resolution of AIHA

#### **Thymoma: Recommendations**

- **If acute treatment is required, first line therapy is prednisolone 1 mg/kg/day (2C)**
- **In all cases, consider surgical resection (2C)**

#### ***Malignant conditions***

Only 1–2% of secondary AIHA is associated with solid organ malignancy (Spira & Lynch, 1979) and the primary tumour site and histology varies. AIHA coinciding with presentation of a malignant tumour is less frequently steroid responsive than idiopathic AIHA (Puthenparambil *et al*, 2010; Spira & Lynch, 1979). Sustained resolution of AIHA has been reported with resection of isolated ovarian, renal cell and colonic carcinomas (Lands & Foust, 1996; Spira & Lynch, 1979) and with chemotherapy ± splenectomy for seminoma (Canale *et al*, 1975; Herve *et al*, 2007; Lundberg & Mitchell, 1977). In metastatic disease, AIHA can respond to disease control or to corticosteroids.

## **INFECTION**

### **Mycoplasma and viral pneumonia**

Although secondary CHAD is a rare complication of mycoplasma infection, atypical or mycoplasma pneumonia accounted for 33% (23/70) of all CHAD patients in one series (Dacie & Worlledge, 1969). Influenza A has also been associated (Dacie, 1962; Schoindre *et al*, 2011) but a pathogen is not always identified. CHAD typically occurs 2–3 weeks after onset of the illness. Acrocyanosis, haemoglobinuria or gangrene are uncommon and haemolysis typically resolves after a further 2–3 weeks (Petz & Garratty, 1980). Most patients can be managed supportively with antibiotics (if unresolved pneumonia), warmth and transfusion for symptomatic anaemia. Some patients have also received corticosteroids or immunoglobulins, although whether they influence the acute course of haemolysis is unknown.

### **CHAD secondary to atypical and mycoplasma pneumonia:**

#### **Recommendations**

- **Treat supportively with appropriate antimicrobials, a warm environment and transfusion for symptomatic anaemia (1C)**
- **If haemolysis is severe and persistent, consider emergency treatment e.g. steroids or immunoglobulins (2C)**

### **Infectious mononucleosis (IM)**

AIHA occurs in up to 3% of patients with IM, typically within 1–2 weeks of onset. Patients present with sore throat, fever and malaise followed by weakness and jaundice. Lymphadenopathy and hepatosplenomegaly are common (Petz & Garratty, 1980). IM is classically associated with an anti-i cold agglutinin with high thermal amplitude (Wilkinson *et al*, 1973). Most cases are self-limiting

within 4–8 weeks. Some benefit has been reported in patients treated with steroids (Bowman *et al*, 1974;Keyloun & Grace, 1966;Tonkin *et al*, 1973) and with plasma exchange in a steroid-refractory case (Geurs *et al*, 1992).

### **Infectious mononucleosis: Recommendations**

- **Patients with mild haemolysis can be monitored for resolution (1C)**
- **If haemolysis is more severe, consider prednisolone 1 mg/kg/day (2C)**
- **If AIHA due to a cold antibody, the patient should avoid cold exposure (2C)**

### **Hepatitis C**

In cases where AIHA is thought to be secondary to interferon, this should be discontinued, but in severe interferon associated cases, steroids have been employed prior to resolution of haemolysis. In eradication treatment naive cases, 15/16 patients had a complete response to first line prednisolone 0.5–2 mg/kg/day (two additionally received cyclophosphamide or azathioprine) (Ramos-Casals *et al*, 2003) and two steroid-refractory patients responded to rituximab 375 mg/m<sup>2</sup> weekly for 4 weeks (Annicchiarico *et al*, ;Etienne *et al*, 2004). However viral load can increase in patients receiving steroids and the main reported cause of death is liver failure. Hepatitis C eradication should therefore also be considered.

### **Hepatitis C: Recommendations**

- **If interferon-induced DIIHA is suspected, discontinue interferon (1A). Consider steroids for severe persistent haemolysis (2C)**
- **In hepatitis C eradication treatment naive AIHA, first line treatment is prednisolone (2C)**
- **In cases of controlled AIHA, consider hepatitis C eradication (2C)**

## IMMUNE DYSREGULATION

### Systemic lupus erythematosus (SLE)

A positive DAT is present in 18–65% of patients (Giannouli *et al*, 2006) while AIHA occurs in 5–10%. Approximately two thirds of cases occur at SLE presentation but AIHA can also present first.

Initial steroid treatment results in a 75–96% response rate (Gomard-Menesson *et al*, 2006; Pirofsky & Bardana, Jr., 1974) and the recurrence rate, estimated at 3–4 per 100 patient years (Gomard-Menesson *et al*, 2006; Kokori *et al*, 2000), appears lower than primary warm AIHA. Where AIHA is the dominant feature, case reports suggest that oral immunosuppressants such as azathioprine (Gomard-Menesson *et al*, 2006) or mycophenolate mofetil (Alba *et al*, 2003; Gomard-Menesson *et al*, 2006) may be useful agents. Danazol can also act as a steroid-sparing agent and the reported response rate was 60% in a series of 15 patients with secondary AIHA, 12 of whom had SLE (Ahn, 1990). Rituximab, which appears beneficial in refractory lupus (Lan *et al*, 2012), has been successfully used in a small number with AIHA, including complete remission in 4/4 paediatric patients (Kumar *et al*, 2009).

In a series of patients undergoing splenectomy, 3/3 patients responded but 2 responses were partial requiring additional medical therapy (Akpek *et al*, 1999). In other studies, only 1/4 (Gomard-Menesson *et al*, 2006) and 0/2 (Videbaek, 1962) achieved sustained responses. In a comparison of 15 SLE patients with ITP and/or AIHA treated medically vs. 15 who received splenectomy, the frequency of cytopenias was the same but splenectomised patients had significantly more serious infections (18 vs. 2) including 2 infection-related deaths (Rivero *et al*, 1979). Splenectomy should therefore be reserved for failure of medical management.

## **SLE where AIHA is the predominant feature: Recommendations**

**First line: steroids (1B)**

**Second line: azathioprine, danazol, mycophenolate mofetil, rituximab (2C)**

### **Common variable immunodeficiency**

Immune dysregulation, leading to autoimmunity (especially immune cytopenias) is a common manifestation of primary immunodeficiencies. Common variable immunodeficiency (CVID) is the most frequent clinically expressed primary immunodeficiency in adults. AIHA occurs in 4–7% of CVID patients. Fewer patients appear to develop immune cytopenias while receiving maintenance immunoglobulin therapy (Wang & Cunningham-Rundles, 2005). Therapy similar to primary warm AIHA can be considered, although lower doses and shorter treatment periods have been recommended (Cunningham-Rundles, 2008). Steroids are usually effective first line therapy and in one series, 6/9 patients required no further treatment (Wang & Cunningham-Rundles, 2005). In another, 15/18 (83%) responded although only 4 had a sustained complete response (Seve *et al*, 2008). Of 12 patients (5 AIHA; 7 Evans syndrome) receiving rituximab (majority 375 mg/m<sup>2</sup> weekly for 4 weeks), 10/12 responded (7/12 complete responses) and 4/8 responding adults maintained their response at 17–105 months. Four had severe infections (Gobert *et al*, 2011). AIHA responded to splenectomy in 4/6 (Resnick *et al*, 2012), 3/5 (Wong *et al*, 2013) and 7/7 patients (Seve *et al*, 2008). In the latter study, 4/7 relapsed after a mean 14 years follow up.

Post-splenectomy infection from encapsulated bacteria is a particular concern in CVID patients and in one study 5/12 developed life threatening infection with *Streptococcus pneumoniae* and/or *Neisseria meningitides* (Seve *et al*, 2008). All had discontinued prophylactic penicillin. In the largest study, 9/40 CVID patients developed bacterial meningitis or pneumococcal sepsis post-splenectomy (Wong

*et al*, 2013). Seven episodes were within 3 years of splenectomy and 7/9 were not on IVIg replacement.

### **CVID: Recommendations**

- **Therapy**
  - **First line: steroids (1B)**
  - **Second line: rituximab (2C)**
  - **Third line: immunosuppression, splenectomy (2C)**
- **Patients receiving steroids, immunosuppression or splenectomy should also receive maintenance IVIg (1C)**
- **Patients who require splenectomy should receive life long prophylactic antibiotics (1B)**

### **Ulcerative colitis**

AIHA is rare in Crohn's disease but occurs in 0.5–0.7% of patients with ulcerative colitis (Gumaste *et al*, 1989;Lakatos *et al*, 2003;Snook *et al*, 1989). AIHA almost always occurs in the presence of active colitis (Ramakrishna & Manoharan, 1994) and control of colitis appears central to management. CMV reactivation, common in such patients (Mowat *et al*, 2011) and associated with AIHA (Salloum & Lundberg, 1994) should be excluded. Results with steroids as a single agent are disappointing, with remission of AIHA and colitis in only 5/18 (28%) patients, rising to 7/16 (44%) if combined with immunosuppressive therapy (Ramakrishna & Manoharan, 1994). Immunosuppression is most frequently a thiopurine, with successful use of ciclosporin (Molnar *et al*, 2003) or infliximab (Leo *et al*, 2009) reported in refractory cases. Resolution of both AIHA and colitis occurred in 10/10 patients following colectomy and 6/6 following colectomy with splenectomy (Ramakrishna & Manoharan, 1994). Isolated splenectomy achieved remission of AIHA in 4/9 patients (Hernandez *et al*, 1994;Ramakrishna & Manoharan, 1994) but does not treat colitis and the expected durability of remission is unclear.

## **Ulcerative colitis: Recommendations**

- **Patients should be managed in conjunction with a gastroenterologist (1C)**
- **Patients with warm AIHA and active colitis (mild or moderate) should receive first line: 1) oral prednisolone and 2) an immunosuppressive agent such as azathioprine (1C)**
- **In refractory cases of warm AIHA with active colitis, aim to control active colitis under the care of a gastroenterologist based on current British Society of Gastroenterology best practice guidelines ([www.bsg.org.uk](http://www.bsg.org.uk)) (2C)**
- **Patients with colitis that is poorly controlled or refractory to medical therapy may require panproctocolectomy. This controls both colitis and AIHA such that concomitant splenectomy is not indicated (2C).**
- **If active colitis has been actively excluded, consider treatment as for primary AIHA (2C)**

## **Haematopoietic stem cell transplantation (HSCT)**

Following allogeneic HSCT, 2–4% of patients develop AIHA after a median time of 3-10 months. Alloimmune haemolysis can usually be distinguished by its early presentation, the presence of ABO mismatch or the antibody specificity. Less frequently, alloimmune haemolysis may present later due to mixed chimaerism and blood group disparity (Kordes *et al*, 2008; Sokol *et al*, 2002). Serological investigation is aided by undertaking extended red cell typing of donor and recipient prior to transplantation. Alternatively samples stored following HLA typing can be retrieved for extended red cell genotyping if auto- and panagglutinins occur and specifically ruling out allo-antibodies of donor or recipient origin would be useful. Transplant-associated thrombotic microangiopathy and DIIHA can occur with calcineurin inhibitors (O'Brien *et al*, 2004) and should be considered in the differential. AIHA may coincide with CMV reactivation, onset of GVHD or relapsing disease (Chen *et al*, 1997).

## ***Treatment***

AIHA resolves in less than half of patients treated with steroids and multiple agents are often required. Rituximab is an effective agent and in one series, 8/9 patients responded to steroids and rituximab given first line and 4/5 responded to rituximab second line (Daikeler *et al*, 2013). In other series, rituximab first or second line resulted in CR in 8/8 patients (Faraci *et al*, 2014), and combined with prednisolone or other immunosuppression second or third line resolved AIHA in 6/13 (Wang *et al*, 2015). Other immunosuppressants such as azathioprine, mycophenolate mofetil, cyclophosphamide, alemtuzumab as well as immunoglobulins and plasma exchange have been used but it is often unclear which treatment the patient responded to (Holbro *et al*, 2012). Response to splenectomy was reported in 5/13 (38%) patients from 4 series (Chen *et al*, 1997;Cwynarski *et al*, 2001;Drobyski *et al*, 1996;Wang *et al*, 2015). Severity of AIHA is variable and although some patients die of refractory disease, most deaths are secondary to infection, relapse or GVHD.

### **HSCT: Recommendations**

- **Consider pre-transplant storage of DNA from donor and recipient for genotyping in the event of the development of auto- and panagglutinins (2C)**
- **At presentation of AIHA, re-evaluate chimaerism and remission status. Assess for and treat infection and GVHD (1C)**
- **Consider switching GVHD prophylaxis (2C)**
- **First line: steroids (2C)**
- **Second line: rituximab (2C)**

### **Solid organ transplantation**

AIHA appears rare in adults but occurs in 5–10% of children following liver and/or intestinal transplantation (Botija *et al*, 2010;Czubkowski *et al*, 2011;Li *et al*, 2012) and 2.5% following pancreas transplantation (Elimelakh *et al*, 2007). Unlike alloimmune haemolysis, which usually presents within the first few weeks, AIHA tends to present months or even years after the transplant. Underlying causes such as CMV infection, parvovirus B19 infection and Epstein–Barr virus (EBV)-associated post transplant lymphoproliferative disorder (PTLD) should be identified. The evidence base for treatment is limited. Post-transplant immune suppression (e.g. tacrolimus) is often reduced or switched, but the importance of this is debated (Li *et al*, 2012). First line therapy is usually steroids although response rates appear lower than in primary AIHA (Botija *et al*, 2010;Li *et al*, 2012). Rituximab for relapsed or refractory AIHA resulted in remission for 3/4 (Li *et al*, 2012) and 3/5 (Botija *et al*, 2010) paediatric patients.

## **EVANS SYNDROME**

Evans syndrome is an uncommon disorder in which there is autoimmune thrombocytopenia (ITP) and autoimmune haemolytic anaemia either occurring at the same time, or consecutively. Neutropenia is also a common feature, present in around 55% at presentation (Mathew *et al*, 1997;Pui *et al*, 1980;Wang, 1988). The disease is generally chronic and affects both children and adults (Pui *et al*, 1980).

Although Evans' original description was of an acquired haemolytic anaemia and primary ITP (Evans & DUANE, 1949;Evans *et al*, 1951), this combination of immune cytopenias can also be secondary to an underlying disorder. In a review of 68 adults, 50% were secondary, mostly to immunodeficiency, collagen vascular disorders or haematological malignancy (Michel *et al*, 2009). Evans syndrome may also develop following stem cell transplantation, drugs or infection. Another important cause of secondary Evans syndrome is autoimmune

lymphoproliferative syndrome (ALPS) (Teachey *et al*, 2010), especially in children.

Because of its rarity, the precise incidence and prevalence are not known. In one series of AIHA and ITP cases, only 4% had primary Evans syndrome (Pui *et al*, 1980). The features seen are those expected in ITP or AIHA and include lethargy, jaundice, shortness of breath, petechiae, bruising or mucocutaneous bleeding. Unlike ITP, clinical examination may reveal hepatosplenomegaly. Lymphadenopathy is suggestive of an associated disorder. Important differential diagnoses in suspected Evans syndrome include: paroxysmal nocturnal haemoglobinuria, disseminated intravascular coagulation, liver disease, acquired or inherited thrombotic thrombocytopenic purpura, haemolytic uraemic syndrome and Kasabach–Merritt syndrome. The approach to investigation is similar to that for AIHA but all patients should be tested for ALPS by flow cytometry on peripheral blood for T-cell subsets. Bone marrow examination may help exclude infiltration in patients with pancytopenia.

### **The management of Evans syndrome**

Data for the management of Evans syndrome are limited to case reports and retrospective studies with small numbers of patients (Norton & Roberts, 2006). It is also not always clear which cytopenia treatment was initially started for. Treatment for secondary Evans syndrome will depend on the underlying disorder and must be individualised.

#### ***First line treatment***

***Corticosteroids*** Corticosteroids are the mainstay of first line therapy (Pui *et al*, 1980;Wang, 1988). Paediatric studies using prednisone 1–2 mg/kg/day resulted in remission in 5 of 7 children (Pui *et al*, 1980). In adults, the same dose

achieved an 83% (53/64) response rate when given for AIHA and 82% for ITP (Michel *et al*, 2009). Unfortunately, the majority of patients relapse.

*Intravenous immunoglobulin* This is useful for patients with Evans syndrome failing to respond to corticosteroids or requiring high doses of corticosteroids to remain in remission. In responders one or more of the cytopenias may correct. Short term responses have been reported in 60–87% (Mathew *et al*, 1997; Michel *et al*, 2009). Most patients receive 2 g/kg in divided doses (Norton & Roberts, 2006) although successful treatment of AIHA has been reported with a higher dose of 1 g/kg for 5 days (Hilgartner & Bussel, 1987).

### ***Second line treatment***

The treatments used are similar to those for AIHA and ITP and include ciclosporin, mycophenolate mofetil, azathioprine, vincristine (for ITP), danazol, rituximab and splenectomy. Most data for immunosuppressants in Evans syndrome are anecdotal and single centre (Kotb *et al*, 2005; Mathew *et al*, 1997). For patients who fail to respond to single agent immunosuppressants, multiagent treatment has been shown to be of some value (Chemlal *et al*, 1999; Scaradavou & Bussel, 1995).

*Azathioprine and other thiopurines* In larger series, 9–14% of patients received azathioprine (Mathew *et al*, 1997; Michel *et al*, 2009) but response rates were not reported and evidence of effectiveness remains anecdotal for azathioprine (Goebel *et al*, 1974) or mercaptopurine (Lyu *et al*, 1986; Tattersall, 1967).

*Ciclosporin* Variable doses and responses have been reported from 0.5–10 mg/kg/day (Emilia *et al*, 1996; Rackoff & Manno, 1994; Ucar *et al*, 1999; Williams & Boxer, 2003). Response rates when combined with corticosteroids and danazol reached 89% (Liu *et al*, 2001).

*Danazol* Limited published data suggest that danazol may be a useful steroid-sparing agent in Evans syndrome (Scaradavou & Bussel, 1995;Wang, 1988). In a retrospective multicentre survey, a 60% response rate was reported in 23 adult patients (Michel *et al*, 2009) but danazol may be less well tolerated in children (Norton & Roberts, 2006).

*Mycophenolate mofetil* This immunosuppressant has been used in Evans syndrome (Guirat-Dhouib *et al*, 2010;Hou *et al*, 2003;Howard *et al*, 2002;Kotb *et al*, 2005). The number of patients treated is small and it is difficult to estimate the response rates.

*Rituximab* This has been shown to be of value in several autoimmune diseases and has been used successfully in Evans syndrome. Doses used range from 100 mg weekly for 4 weeks to 375 mg/m<sup>2</sup> weekly for up to 4 weeks. Response rates were 13/17 (Bader-Meunier *et al*, 2007), 9/11 (Michel *et al*, 2009), 5/5 (Zecca *et al*, 2003) and 2/4 (Shanafelt *et al*, 2003).

*Splenectomy* The response rates for splenectomy are lower than those seen in ITP, at less than 70% but data are limited (Blanchette & Price, 2003). Responses are sometimes transient with relapses seen at 1–2 months post-splenectomy (Mathew *et al*, 1997;Pui *et al*, 1980;Wang, 1988). However in one series, 52% (10/19) maintained a response at a mean follow-up of 8 years (Michel *et al*, 2009). Splenectomy is best avoided in children under the age of 6 years (Norton & Roberts, 2006) but should be considered in older children and adults if other treatments fail.

*Vincristine* This is useful for treating the thrombocytopenia in Evans syndrome (Wang, 1988). From available data, vincristine looks more useful when combined with other agents (Scaradavou & Bussel, 1995;Williams & Boxer, 2003).

### ***Treatment options for patients failing second line therapies***

Again, data are very limited but third line agents have included cyclophosphamide, alemtuzumab and stem cell transplantation. Cyclophosphamide has been used at 1–2 mg/kg orally (Gombakis *et al*, 1999; Oda *et al*, 1985; Wang, 1988). Alemtuzumab has been used successfully in a few cases (Willis *et al*, 2001). Stem cell transplantation (autologous and allogeneic) has been reported in a few patients. Although the numbers are small, in one group of patients 50% were alive and in complete remission (Hough *et al*, 2005; Huhn *et al*, 2003; Passweg *et al*, 2004; Raetz *et al*, 1997).

### ***Novel therapies***

The thrombopoietin receptor agonist, romiplostim, has been used successfully in a patient with Evans syndrome to elevate the platelet count (Gonzalez-Nieto *et al*, 2011). Currently the drug is not approved for use in Evans syndrome but is likely to be useful in this setting.

### **Primary Evans syndrome: Recommendations**

- **First line treatment: Corticosteroids, IVIg (1C)**
- **Second line treatment: azathioprine, ciclosporin, danazol, mycophenolate mofetil, rituximab, splenectomy, vincristine (ITP) (2C)**

### **CHILDHOOD AIHA ASSOCIATED WITH GIANT CELL HEPATITIS (GCH)**

GCH is a histological finding, more commonly seen in neonates with cholestasis. When associated with AIHA, GCH usually presents between 2 months and 2 years of age with jaundice, hepatomegaly, elevated conjugated bilirubin and alanine aminotransferase. GCH typically presents simultaneously with a DAT positive (IgG + C) warm AIHA but in a third of cases AIHA presents first (Maggiore *et al*, 2011).

AIHA associated with GCH is usually severe and relapsing. Unless there is acute refractory liver failure requiring transplantation, treatment of both GCH and AIHA is with immunosuppression. Initial treatment has usually been prednisolone and azathioprine, but sustained remission is uncommon. Hepatic injury appears to be B-cell mediated and from two series, 8/8 treatment refractory patients responded to 375 mg/m<sup>2</sup> rituximab weekly for 3-5 weeks (Bakula *et al*, 2014;Paganelli *et al*, 2014).

### **Childhood AIHA with giant cell hepatitis: Recommendations**

- **Unexplained elevated hepatic transaminases should lead to the consideration of giant cell hepatitis and liver biopsy (2C).**

### **DRUG-INDUCED IMMUNE HAEMOLYTIC ANAEMIA (DIIHA)**

The incidence of DIIHA is approximately 1 per million/year (Garratty, 2010). Over 130 individual drugs have been implicated but the most commonly reported include second and third generation cephalosporins, diclofenac, rifampicin, oxaliplatin and fludarabine (Salama, 2009) (see also table II and [supplementary appendix 2](#) for a detailed list). Therapeutic IVIg can also cause acute haemolysis related to passive transfer of antibodies e.g. to ABO or Rh antigens. Some drugs (e.g. fludarabine, cladribine, levodopa, mefenamic acid and procainamide) cause drug-independent DIIHA that can be serologically indistinguishable from warm AIHA while others can only be detected *in vitro* in the presence of the drug or its metabolites (drug-dependent DIIHA).

Patients can present within hours of exposure to drug with severe complement-mediated intravascular haemolysis (e.g. ceftriaxone) or sub-acutely with extravascular haemolysis after several months of exposure. Fatality rates of 6–15% have been reported with cephalosporins and diclofenac (Ahrens *et al*, 2006;Garratty, 2010). Acute intravascular DIIHA can be mistaken for a

haemolytic transfusion reaction or acute sepsis and the drug history should include perioperative antibiotics and over the counter analgesia (e.g. NSAIDS).

## **Investigations**

The DAT is almost always positive for IgG and/or C3 (unless massive intravascular haemolysis has occurred or red cell transfusion has been given prior to testing) (Garratty, 2010). Warm AIHA is more common than DIIHA and further investigation is only required if there is clear evidence of haemolysis and a good temporal relationship with the suspected drug. Serological investigation is not indicated if the suspected drug is known to be associated with drug-independent haemolysis (e.g. fludarabine). Investigation should be undertaken by an experienced red cell reference laboratory. The laboratory should be consulted about appropriate samples (e.g. patient's blood, sample of suspected medication, urine from patient or volunteer taking same medication, for metabolites).

## **Management**

The suspected drug should be stopped and haematological improvement usually occurs in 1–2 weeks. In patients with acute severe DIIHA, establish intravenous access and commence fluid resuscitation. Monitor vital signs, urine output, renal function and haemoglobin. Patients may require an intensive care environment and temporary dialysis. Approximately 55% of patients with DIIHA will require blood transfusion (Garbe *et al*, 2011). The addition of steroids is of uncertain benefit and any influence is hard to distinguish from the effects of stopping the drug, however in one study 105/124 (85%) patients received corticosteroids (Garbe *et al*, 2011).

### **DIIHA: Recommendations**

- **Discontinue the suspected drug (1A)**

- **When DIIHA is suspected, liaise early with the local red cell immunohaematology reference centre to determine appropriate investigations (1C)**
- **The benefit of corticosteroids is unclear. The decision whether to start corticosteroids must be individualised and will depend on the severity of haemolysis and strength of clinical suspicion that haemolysis is drug induced (2C).**

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## **AUTHOR CONTRIBUTIONS**

All authors were involved in formulation, writing and approval of the guidelines. All authors approved the final version of the manuscript.

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## **DECLARATION OF INTERESTS**

All authors have made a full declaration of interests to the BCSH and Task Force Chairs, which may be reviewed on request. The following members of the writing group have no conflicts of interest to declare: QAH, RS, EM, DP, JDG and AH.

## **REVIEW PROCESS**

Members of the writing group will inform the writing group Chair if any new pertinent evidence becomes available that would alter the strength of the recommendations made in this document or render it obsolete. The document

will be archived and removed from the BCSH current guidelines website if it becomes obsolete. If new recommendations are made an addendum will be published on the BCSH guidelines website at [www.bcsguidelines.com](http://www.bcsguidelines.com). If minor changes are required due to changes in level of evidence or significant additional evidence supporting current recommendations a new version of the current guidance will be issued on the BCSH website.

## **DISCLAIMER**

While the advice and information in these guidelines is believed to be true and accurate at the time of going to press, neither the authors, the British Committee for Standards in Haematology (BCSH) nor the publishers accept any legal responsibility for the content of these guidelines.

## Tables and figures

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### Warm AIHA

Primary

Secondary

Neoplasia (CLL, lymphoma, solid organ)

Infection (e.g. hepatitis C, HIV, CMV, VZV, pneumococcal infection, leishmaniasis, tuberculosis)

Immune dysregulation

Connective tissue disorders (e.g. SLE, Sjögren's syndrome, scleroderma)

Ulcerative colitis, PBC, sarcoidosis

Post transplantation

Immune deficiency syndromes (e.g. CVID)

### Cold AIHA

Cold haemagglutinin disease

Primary

Secondary

Malignancy (e.g. CLL, NHL, solid organ)

Infection (e.g. mycoplasma, viral infections including IM)

Autoimmune disease

Post-allogeneic HSCT

Paroxysmal cold haemoglobinuria

Primary

Secondary

Infection (e.g. adenovirus, influenza A, syphilis, CMV, IM, VZV, measles, mumps, *Mycoplasma pneumoniae*, *Haemophilus influenzae*, *E. coli*)

### Mixed type AIHA

Primary

Secondary

Lymphoma, SLE, infection

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### Table I. Classification of autoimmune haemolytic anaemia

AIHA, autoimmune haemolytic anaemia; CLL, chronic lymphocytic leukaemia; CMV, cytomegalovirus; CVID, common variable immunodeficiency; HIV, human immunodeficiency virus; HSCT, haematopoietic stem cell transplantation; IM, infectious mononucleosis; NHL, non-Hodgkin lymphoma; PBC, primary biliary cirrhosis; SLE, systemic lupus erythematosus; VZV, varicella zoster virus..

<b>Drug</b>	<b>Cases<sup>†</sup></b>	<b>Cases*</b>
Anti-infectives		
Ceftriaxone	3	17
Other cephalosporins		37
β-lactamase inhibitors		6
Piperacillin	3	14
Ciprofloxacin	3	
Doxycyclin	2	
Amoxicillin	2	
Cotrimoxazole	2	
Influenza vaccine	2	
Other	14	3
Antineoplastics		
Fludarabine	6	
Oxaliplatin	3	3
Chlorambucil	2	
Other	3	1
Musculoskeletal		
Diclofenac	14	1
Paracetamol	3	
Ibuprofen	2	
Other	3	
Cardiovascular		
Hydrochlorothiazide	3	
Amlodipine	3	
Ramipril	3	
Enalapril	2	
Other	10	
Alimentary		
Omeprazole	2	
Other	2	1
Miscellaneous	12	
<b>Total</b>	<b>104</b>	<b>84</b>

**Table II. Distribution of cases of drug induced haemolytic anaemia in 2 major series**

† (Garbe 2011), \* (Garratty 2010). Both presented 10-year data (2000-2009). In the series of Garratty, 36 cases (43%) were due to cefotetan, an antibiotic not available in the United Kingdom.

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## **Supplementary appendix 1: systematic review methodology for the 2016 BCSH guideline on the management of drug-induced immune and secondary autoimmune, haemolytic anaemia.**

The first systematic review of Medline and Embase was from 1960 to 24/01/2012 (search strategy is outlined below). The search was limited to English language peer reviewed publications and conference abstracts were not systematically reviewed. All 4316 Medline and 6454 Embase titles were reviewed for relevance. The DARE database was searched in August 2012 using the MeSH descriptor terms anaemia, autoimmune and haemolytic. The COCHRANE database of systematic reviews was also searched in August 2012. Some key studies published prior to 1960 were also been reviewed. The main search was also supplemented by searches of Pubmed in which the search term "autoimmune hemolytic anemia" was combined with specific disorders e.g. "thymoma" or "ulcerative colitis".

The systematic review of Medline and Embase was updated 15/11/2015 using the same search strategy. The DARE database and the COCHRANE database of systematic reviews were also searched but no new documents identified.

### Medline search (24/01/2012)

1. exp Anemia, Hemolytic, Autoimmune/
2. limit 1 to (english language and yr="1960 -Current")
3. autoimmune h?emolytic an?emia.mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
4. limit 3 to (english language and yr="1960 -Current")
5. (autoimmune adj3 an?emia).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
6. limit 5 to (english language and yr="1960 -Current")
7. cold agglutinin?.mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
8. limit 7 to (english language and yr="1960 -Current")
9. 2 or 4 or 6 or 8 (number of documents retrieved = 4316)
10. limit 9 to "all child (0 to 18 years)" (number of documents retrieved = 960)

### Embase search (24/01/2012)

1. autoimmune h?emolytic an?emia.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
2. limit 1 to (english language and yr="1960 -Current")

3. (autoimmune adj3 an?emia).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
4. limit 3 to (english language and yr="1960 -Current")
5. cold agglutinin?.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
6. limit 5 to (english language and yr="1960 -Current")
7. exp autoimmune hemolytic anemia/
8. limit 7 to (english language and yr="1960 -Current")
9. 2 or 4 or 6 or 8 (number of documents retrieved = 6454)
10. limit 9 to child (number of documents retrieved = 542)

**Supplementary appendix 2: Drugs associated with cases of immune haemolytic anaemia (IHA), positive direct antiglobulin test (DAT) or both.**

1) Drugs associated with cases of IHA or positive DAT or both in which drug-dependent antibodies were detected.

Table adapted from: An update on drug-induced immune hemolytic anemia. Garratty G & Arndt PA *Immunohematology* 2007;23(3):105-19 with permission of the American National Red Cross

<b>Drug (Alternative name)</b>	<b>Therapeutic category</b>	<b>Number of references [single (year) vs. multiple (&lt;5, &lt;10, ≥10)]</b>
Aceclofenac	NSAID	Single (1997)
Paracetamol (Acetaminophen)	NSAID	Multiple (<10)
Aciclovir	Antiviral	Single (2003)
Aminopyrine (Piramidone)	NSAID	Single (1961)
Amoxicillin	Antimicrobial	Single (1984)
Amphotericin B	Antimicrobial	Multiple (<5)
Ampicillin	Antimicrobial	Multiple (<10)
Antazoline	Antihistamine	Multiple (<5)
Aspirin	Analgesic, antipyretic, anti-inflammatory	Single (1984)
Azapropazone (Apazone)	Antiinflammatory, analgesic	Multiple (<5)
Buthiazide (Butizide)	Diuretic, antihypertensive	Single (1984)
Carbimazole	Antithyroid	Multiple (<5)
Carboplatin‡	Antineoplastic	Multiple (<5)
Carbromal	Sedative, hypnotic	Single (1970)
Catechin ((+)-Cyanidanol-3) (Cianidanol)	Antidiarrheal	Multiple (≥10)
Cefamandole	Antimicrobial	Single (1985)
Cefazolin	Antimicrobial	Multiple (<10)
Cefixime	Antimicrobial	Single (2000)
Cefotaxime‡	Antimicrobial	Multiple (<5)
Cefotetan‡	Antimicrobial	Multiple (≥10)
Cefoxitin‡	Antimicrobial	Multiple (<10)
Cefpirome	Antibacterial	Single (2005)
Ceftazidime	Antimicrobial	Multiple (<10)
Ceftizoxime	Antimicrobial	Multiple (<5)
Ceftriaxone‡	Antimicrobial	Multiple (>10)
Cefuroxime	Antibacterial	Multiple (<5)
Cephalexin	Antimicrobial	Multiple (5)
Cephalothin‡	Antimicrobial	Multiple (≥10)
Chloramphenicol	Antibacterial	Multiple (<5)
Chlorinated hydrocarbons	Insecticides	Multiple (<10)
Chlorpromazine	Antiemetic, antipsychotic	Multiple (<10)
Chlorpropamide‡	Antidiabetic	Multiple (<10)
Ciprofloxacin	Antibacterial	Multiple (<10)
Cisplatin (Cisdiamino-dichloroplatinum)	Antineoplastic	Multiple (<10)
Cloxacillin	Antibacterial	Single (1980)

Cyclofenil	Gonad-stimulating principle	Multiple (<5)
Ciclosporin (Cyclosporine)	Immunosuppressant	Multiple (<5)
Dexchlorpheniramine maleate (Chlorpheniramine)	Antihistaminic	Single (1981)
Diclofenac‡	NSAID	Multiple (≥10)
Diethylstilbestrol (Stilboestrol)	Estrogen	Multiple (<5)
Dipyron	NSAID	Multiple (<5)
Erythromycin‡	Antimicrobial	Multiple (<5)
Etodolac	NSAID	Single (2000)
Ethambutol	Antibacterial	Single (2003)
Fenoprofen	NSAID	Single (1988)
Fluconazole	Antifungal	Single (2003)
Fluorescein	Injectable dye	Single (1993)
Fluorouracil	Antineoplastic	Multiple (<5)
Furosemide	Diuretic	Multiple (<5)
Glafenine (Glaphenine)	Analgesic	Multiple (<5)
Hydralazine	Antihypertensive	Single (1977)
Hydrochlorothiazide‡	Diuretic	Multiple (<10)
9-Hydroxy-methyl-ellipticinium (Elliptinium acetate)	Antineoplastic	Multiple (<5)
Ibuprofen	NSAID	Multiple (<5)
Imatinib mesylate	Antineoplastic	Multiple (<5)
Insulin	Antidiabetic	Multiple (<5)
Isoniazid	Antimicrobial	Multiple (<10)
Latamoxef (Moxalactam)	Antimicrobial	Single (1985)
Levofloxacin (Ofloxacin)	Antibacterial	Multiple (<5)
Mefloquine‡	Antimicrobial	Multiple (<5)
Melphalan	Antineoplastic	Single (1967)
Mercaptopurine	Anti-neoplastic	Single (2000)
Methadone	Analgesic	Multiple (<5)
Methotrexate	Antineoplastic, antirheumatic	Multiple (<5)
Metrizoate-based radiographic contrast media		Multiple (<5)
Minocycline	Antibacterial	Single (1994)
Nabumetone	Antiinflammatory, analgesic	Single (2003)
Nafcillin‡	Antimicrobial	Multiple (<10)
Naproxen	Antiinflammatory, analgesic, antipyretic	Multiple (<5)
Nitrofurantoin	Antibacterial	Single (1981)
Nomifensine§	Antidepressant	Multiple (≥10)
Norfloxacin	Antimicrobial	Single (1999)
Oxaliplatin‡	Antineoplastic	Multiple (≥10)
<i>p</i> -Aminosalicylic acid (PAS) (para-aminosalicylsäure)	Antimicrobial	Multiple (<10)
Penicillin G‡	Antimicrobial	Multiple (≥10)
Phenacetin‡ (Acetophenetidin)	NSAID	Multiple (≥10)
Phenytoin (Fenitoin)	Anticonvulsant, antiarrhythmic	Single (2003)
Piperacillin‡	Antimicrobial	Multiple (<10)
Probenecid‡	Uricosuric	Multiple (<5)
Propyphenazone	NSAID	Single (1998)
Pyrazinamide	Antibacterial	Single (2003)
Pyrimethamine (Pirimetamine)	Antimicrobial	Multiple (<5)
Quinidine	Antiarrhythmic, antimicrobial	Multiple (≥10)

Quinine	Antimicrobial	Multiple (<10)
Ranitidine	Antiulcerative	Multiple (<5)
Rifabutin	Antibacterial	Single (2003)
Rifampin‡ (Rifampicin)	Antibacterial	Multiple (≥10)
Stibophen	Antimicrobial	Multiple (<5)
Streptokinase	Thrombolytic	Single (1989)
Streptomycin	Antimicrobial	Multiple (<10)
Sulfasalazine	Antiinflammatory	Multiple (<5)
Sulfisoxazole	Antibacterial	Single (2003)
Sulindac	Antiinflammatory	Multiple (<10)
Suprofen	NSAID	Single (1989)
Tartrazine	Colorant	Single (1979)
Teicoplanin	Antimicrobial	Single (2004)
Temafloxacin§	Antimicrobial	Multiple (<5)
Teniposide	Antineoplastic	Single (1982)
Tetracycline	Antimicrobial	Multiple (<10)
Thiopental sodium	Anesthetic	Single (1985)
Ticarcillin‡	Antimicrobial	Multiple (<5)
Tolbutamide	Antidiabetic	Multiple (<5)
Tolmetin‡	NSAID	Multiple (≥10)
Triamterene	Diuretic	Multiple (<5)
Trimellitic anhydride	Used in preparation of resins, dyes, adhesives, etc.	Single (1979)
Trimethoprim and sulfamethoxazole‡	Antibacterial	Multiple (<5)
Vancomycin	Antibacterial	Single (2003)
Zomepirac	NSAID	Single (1983)

IHA = immune haemolytic anaemia; HA = haemolytic anaemia; NSAID = nonsteroidal antiinflammatory drug.

† One or more samples only positive or strongest reactions seen with *ex vivo* (urine or serum) or metabolite.

‡ We have seen cases of drug-induced immune haemolytic anaemia or positive DAT or both attributable to these.

§ No longer manufactured.

2) In the same review, a further 17 drugs were reported as being only associated with drug-independent antibodies. These reports were often based on haemolytic anaemia or a positive DAT or both developing after drug therapy and responding to stopping the drug. Since confirmatory laboratory testing is not possible and patients were rarely re-challenged with the drug, a true relationship between drug and IHA has not always been established.

The drugs reported were: captopril, chaparral, cimetidine, cladribine, fenfluramine, fludarabine, interferon, interleukin-2, ketoconazole, lenalidomide, levodopa, mefenamic acid, mesantoin, methylodopa, nalidixic acid, procainamide and tacrolimus.